# Temperature-Driven Dynamics: Exploring Ciliate Feeding Patterns and Microbial Ecosystem Implications

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**Abstract**

Ciliates, such as *Tetrahymena Thermophila*, are integral components of microbial food webs in freshwater and marine ecosystems, influencing the dynamics of organic matter cycling. This study investigates the temperature-driven dynamics of ciliate feeding patterns and microbial ecosystem implications, focusing on *Tetrahymena Thermophila*. The methods used in the study include cell counting, phagocytosis analysis, and swimming speed measurement. Our results show a strong correlation between temperature and vacuole formation in *T. Thermophila*, as the number of vacuoles increases with temperature, indicating a faster vacuole formation rate. Moreover, the average speed of the ciliates also increases with temperature. We argue that this rise in feeding activity at higher temperatures may lead to more efficient cycling of organic matter through microbial communities, with cascading effects on nutrient availability and trophic interactions within aquatic ecosystems. These findings suggest that shifts in temperature regimes, whether seasonal or due to long-term climate trends, could reshape the structure and function of microbial food webs in aquatic environments. Further research is warranted to grasp the cascading effects of temperature-induced changes in ciliate behavior on broader ecosystem dynamics.

**Keywords:** *Tetrahymena thermophila*, Temperature-dependent feeding patterns, Ciliate behavior, Microbial ecosystems, Microbial dynamics, Climate change, Vacuole formation, Microbial food webs.

## 1 Introduction

Ciliates, such as *Tetrahymena Thermophila*[1], play a vital role in microbial food webs within freshwater and marine ecosystems. Their rapid movement and consumption of smaller microbes contribute to the cycling of organic matter through microbial food webs. In this experiment, we investigate how temperature impacts the feeding patterns of *T. Thermophila*, exploring the implications for environmental processes, climate dynamics, and microbial food webs[2].

## 2 Methods

### 2.1 Cell count

*T. Thermophila* cultures were grown in YPD medium + Fe at three temperature conditions: 5, 20, and 30°C. Cell counts were performed using a Bürker counting chamber, with a depth of 0.1 mm. At least 10 squares per sample were counted, and the mean count was used to calculate cell density (cells $mL^{-1}$) using the formula: cells $mL^{-1}$ = (Mean count × Dilution) / $2.5 \times 10^{-7}$.

### 2.2 Phagocytosis

To evaluate phagocytosis, *T. Thermophila* cultures were mixed with 1% charcoal ink microparticles. Samples were taken at 5, 10, 20, and 30 minutes, and images were captured. Using Fiji from ImageJ,

[1] See Plum et al., *"Experimental Evolution in Tetrahymena"* [1].

[2] See Pham et al., *"The Effect of Temperature on Food Vacuole Formation in Tetrahymena thermophila"* [2], and Luan et al., *"The effect of temperature on food vacuole formation by Tetrahymena thermophila"* [3].

the number of ink-filled vacuoles was determined. We also used Fiji to measure vacuole size, and the data were analyzed to observe changes over time.

### 2.3 Swimming speed

Videos of *T. Thermophila* swimming at different temperatures were recorded and analyzed using Fiji. The "Trackmate" plugin was employed to track cell movement, and the "TRACK_MEAN_SPEED" values were converted to $\mu$m/s for each condition.

## 3 Results

### 3.1 Cell Count

We are sorry to confess that we only managed to recover insufficient data from the different groups with respect to counts and dilution. We were missing either the temperature, the dilution or the count itself. While this is a bit of a setback, we believe that the phagocytosis data and swimming speed data is more insightful and exciting, and hope that it will make up for it!

### 3.2 Phagocytosis

Displayed below are three pairs of boxplots illustrating the evolution of vacuole number (on the left) and vacuole size (on the right) over a 30-minute interval. These three pairs of graphs represent observations under three distinct temperature conditions: 5°C, 20°C, and 30°C.

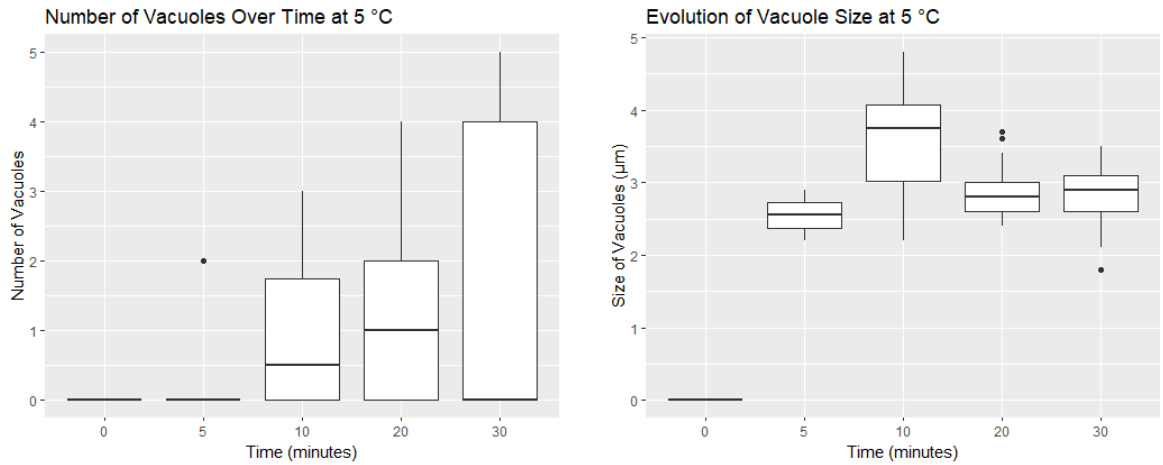


Figure 1: Evolution of vacuole number and size at 5°C. For this particular temperature, a boxplot might not be the clearest plot type since there is a significant proportion of ciliates that do not even have a single vacuole filled with charcoal.

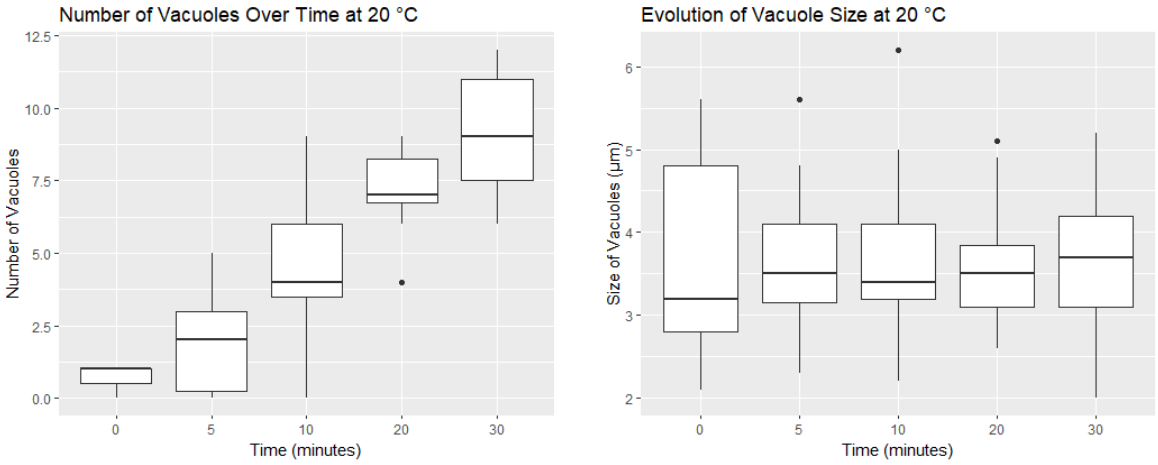


Figure 2: Evolution of vacuole number and size at 20°C

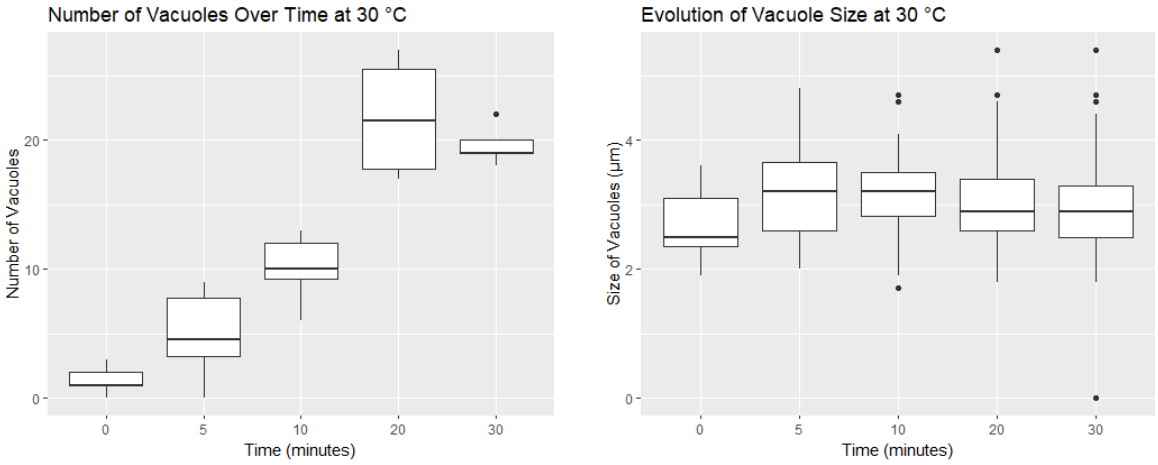


Figure 3: Evolution of vacuole number and size at 30°C

To summarize our findings regarding vacuole number and size, please find below two summary plots, superposing the boxplots for the 3 temperature conditions (from Figures 1, 2, and 3):

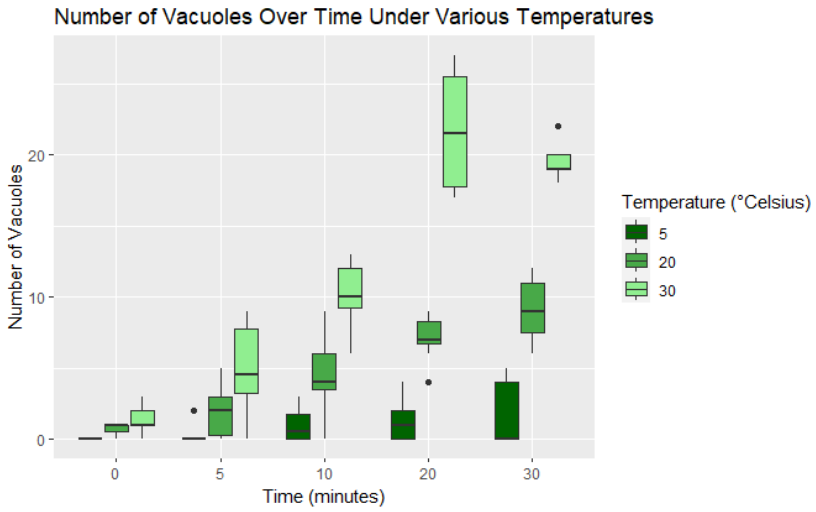


Figure 4: Cumulative plot of the evolution of vacuole number over 30 minutes, under various temperatures

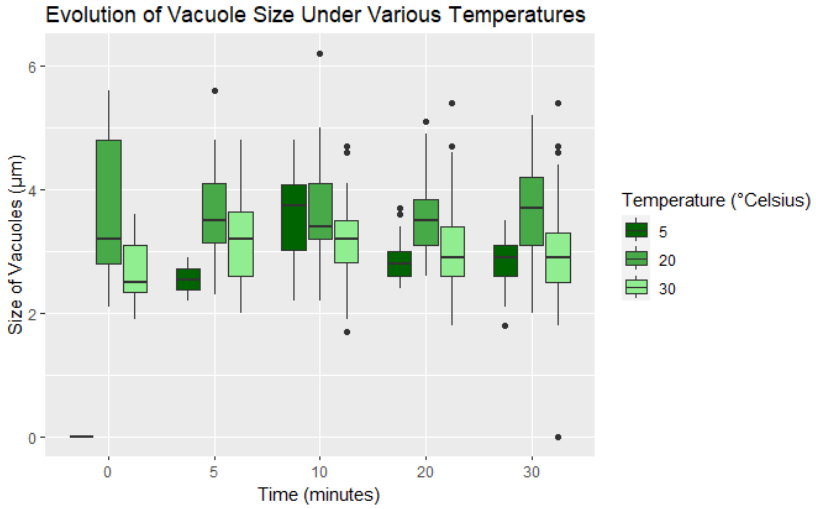


Figure 5: Cumulative plot of the evolution of vacuole size over 30 minutes, under various temperatures

As you can see from the summarized plots above, there is a strong correlation between temperature and vacuole formation in *T. Thermophila*. First, looking at the number of vacuoles observed, we can notice a major trend: the number of vacuoles increases with temperature, underlying the fact that the speed of vacuole formation increases with temperature. Furthermore, there seems to be an optimal number of vacuoles a little below 20, and an optimal size between 3 and 4 microns, and both are reached faster with higher temperatures. This implies that under warmer conditions, *T. thermophila* not only absorbs charcoal particles more rapidly but also organizes its vacuoles more efficiently for storage.

### 3.3 Movement

Below is a box plot of the average speed of the ciliates in the videos recorded at the three different temperatures:

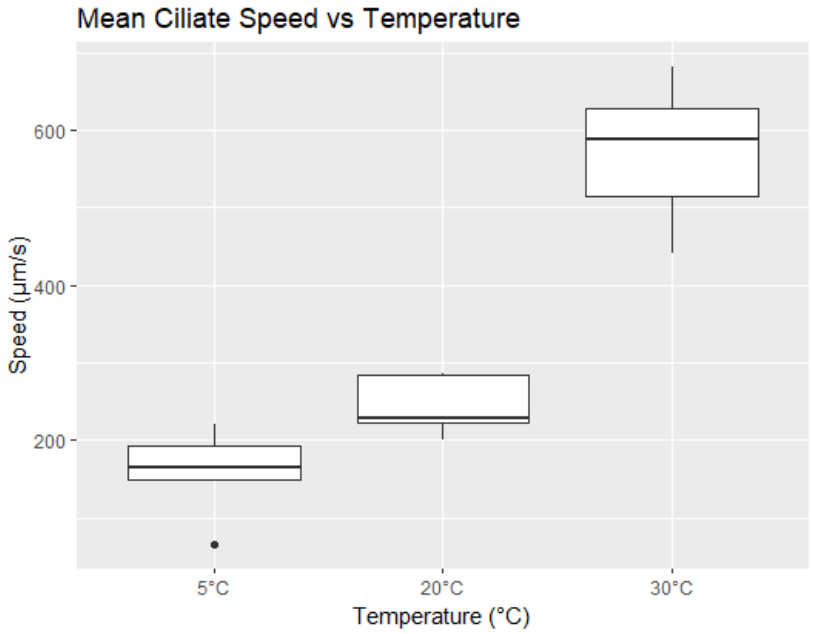


Figure 6: Evolution of mean ciliate speed with temperature. Data was obtained through motion tracking software, as described in the Methods section, and blatant outliers were removed (mainly 0 values due to false positives).

As you can notice, there is a clear positive correlation between the ciliates' speed and the increase in temperature. Also note that the difference in mean speed between 5°C and 30°C is around a three fold increase!

## 4 Discussion

### 4.1 Temperature Influence on Feeding Patterns

The observed increase in vacuole formation over time at higher temperatures aligns with the known physiological responses of *T. thermophila*[3]. As a thermophilic ciliate, *T. thermophila* is adapted to thrive in warmer environments. The faster vacuole formation could be attributed to an accelerated metabolic rate at elevated temperatures, leading to increased phagocytosis and digestion rates. This temperature-dependent response is consistent with the general principle that higher temperatures often enhance metabolic processes in many organisms.

The positive correlation between temperature and feeding patterns has implications for the energy dynamics within microbial food webs. As *T. thermophila* plays a crucial role in the microbial loop, increased feeding activity at higher temperatures may lead to more efficient cycling of organic matter through microbial communities. This enhanced microbial processing could have cascading effects on nutrient availability and trophic interactions within aquatic ecosystems.

### 4.2 Implications for the Environment and Climate

Understanding the temperature-dependent feeding patterns of *T. thermophila* has broader implications for environmental and climate dynamics. With global temperatures on the rise due to climate change, microbial communities in aquatic ecosystems can experience changes in the abundance and activity of

[3]See Weber et al., *"Phenotypic responses to temperature in the ciliate Tetrahymena thermophila"* [4].

ciliates such as *T. thermophila*. The observed temperature-driven increase in feeding rates suggests that climate-induced changes could influence the efficiency of nutrient cycling in aquatic environments.

Moreover, the implications extend beyond *T. thermophila* to the broader microbial food webs. Changes in the feeding patterns of ciliates can impact the abundance and composition of microbial communities, influencing the balance between autotrophic and heterotrophic organisms. This, in turn, may have consequences for higher trophic levels, including fish and other aquatic organisms that rely on microbial communities as a food source.

### 4.3 Temperature Impact on Ciliate Swimming Speed:

The observed increase in swimming speed at higher temperatures further supports the notion that temperature influences the behavior and physiology of *T. thermophila*. The faster swimming speed could be linked to enhanced motility and prey detection strategies, allowing the ciliates to more efficiently locate and capture food particles.

### 4.4 Linking Feeding Patterns to Microbial Food Webs

Temperature-dependent changes in feeding patterns and swimming speed of *T. thermophila* underscore the intricate interplay between environmental temperature and microbial dynamics. The microbial food web is a complex network of interactions, and alterations in the feeding behavior of key ciliate species can reverberate throughout the entire ecosystem.

As temperatures rise, microbial processes may become more dynamic, with potential consequences for nutrient cycling, carbon fluxes, and overall ecosystem stability. The findings suggest that shifts in temperature regimes, whether seasonal or due to long-term climate trends, could reshape the structure and function of microbial food webs in aquatic environments.

## 5 Limits

In the course of our experiment, we encountered several limitations that have a nontrivial impact on our results.

Firstly, one can argue that the quantity of collected data, particularly in the vacuole count phase, is insufficient. The limited number of ciliate pictures per time frame per temperature compromises the statistical power of our analysis. This constraint may have implications for the reliability of observed trends.

Due to data limitations, we were forced to focus our analysis exclusively on the dataset from group 2, which was the only complete dataset available. This selective use of data introduces the possibility of bias and may restrict the applicability of our conclusions to a broader context. Ideally, we wanted to use combined data from all groups, but decided not to for time and bias reasons, as the conditions might have been a little different between the groups.

Moreover, the manual nature of our data extraction process, involving the counting and measurement of vacuoles, introduces potential sources of human error and subjectivity. To enhance accuracy and efficiency, we recommend considering automation of this process in future experiments, utilizing tools such as Napari, to detect and measure vacuoles automatically. This is particularly relevant if we were to increase the size of the dataset, as measuring up to 20 vacuoles per ciliate, multiplied by about 30 ciliates per time frame, per temperature, would be quite tedious.

The temporal resolution of our experiment, particularly in the phagocytosis analysis with intervals of 5, 10, 20, and 30 minutes, may not capture rapid changes in ciliate behavior. Future experiments would benefit from shorter time intervals to provide a more detailed understanding of temporal dynamics.

Additionally, the accuracy of vacuole size measurements using Fiji may be influenced by the resolution of captured images. Consideration should be given to employing higher-resolution imaging systems or advanced microscopy techniques to improve the precision of size measurements. But that probably accounts for a very small part of the imprecision in the data.

Regarding the movement data, we lacked the time and experience to use the rest of the tracking data, which could give us interesting insights, on directionality for example. While playing around

with the given data, I noticed patterns in the length of the straight lines made by the ciliates and how often they turned, but was unable to highlight it in the report.

Finally, the controlled laboratory conditions in which the experiment was conducted may not fully capture potential environmental variability if we want to look at other factors. Future studies could explore the impact of environmental factors, such as light intensity, water chemistry, or nutrient availability, on ciliate behavior for a more comprehensive understanding.

### 5.1 Recommendations for Future Experiments

To address these identified limitations and further our understanding of temperature-driven dynamics in *Tetrahymena thermophila* forward, we propose several strategies for future experiments.

First and foremost, efforts should be made to increase the quantity of collected data, aiming for a more extensive dataset with a higher number of ciliate pictures per time frame per temperature. This expansion would bolster statistical power and provide a more nuanced perspective on temperature-dependent behaviors.

To streamline the data extraction process and reduce potential sources of error, future experiments could benefit from the implementation of automated tools such as Napari. Automation facilitates the analysis of larger datasets while ensuring a more consistent and objective approach.

Consideration should be given to using shorter time intervals in phagocytosis analysis, particularly during the initial stages of temperature-induced changes. This adjustment would allow for a more detailed examination of vacuole formation dynamics. Indeed, in several pictures we can notice cell death, vacuole formation etc. which we could try link to temperature changes.

Lastly, the exploration of additional environmental parameters, such as light conditions or nutrient variations, is recommended. This approach aims to better simulate natural conditions and enhance the ecological relevance of the study, providing a more holistic view of ciliate behavior in aquatic ecosystems.

## 6 Conclusion

All in all, this experiment gives us solid hints regarding the temperature-dependent feeding patterns of *T. thermophila* and their potential consequences for microbial food webs. The observed increase in vacuole formation and swimming speed at higher temperatures highlights the responsiveness of these ciliates to environmental cues. These findings contribute to our understanding of the intricate links between temperature, microbial dynamics, and ecosystem processes in aquatic environments. Our results only emphasize the need for further research, particularly under realistic environmental conditions, to fully elucidate the cascading effects of temperature-induced changes in ciliate behavior on broader ecosystem dynamics.

## Code and Data

While the code is quite rudimentary, it might be of use for further studies, so I made a git repo containing all the data, code, and report used for this project :
https://github.com/Ant-Babu/temperature_ciliate.git

## References


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